GASTROENTEROLOGY 2003;124:1193–1201

CLINICAL–ALIMENTARY TRACT

Increased Risk of Noncardia Gastric Cancer Associated With Proinflammatory Cytokine Gene Polymorphisms

EMAD M. EL–OMAR,*,† CHARLES S. RABKIN,† MARILIE D. GAMMON,§ THOMAS L. VAUGHAN,‖ HARVEY A. RISCH,¶ JANET B. SCHOENBERG,# JANET L. STANFORD,‖ SUSAN T. MAYNE,¶ JAMES GOEDERT,† WILLIAM J. BLOT,** JOSEPH F. FRAUMENI, JR.,† and WONG–HO CHOW†

*Department of Medicine and Therapeutics, Aberdeen University, Aberdeen, Scotland; †Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; §Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina; ‖Program in Epidemiology, Fred Hutchinson Cancer Research Center, and Department of Epidemiology, University of Washington School of Public Health, Seattle, Washington; ¶Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut; #Applied Cancer Epidemiology Program, New Jersey Department of Health and Senior Services, Trenton, New Jersey; and **International Epidemiology Institute, Rockville, Maryland

Background & Aims: **Genetic variations in proinflammatory and anti-inflammatory cytokine genes influence individual response to carcinogenic exposures. Polymorphisms in interleukin (IL)-1β and its endogenous receptor antagonist are associated with risk of *Helicobacter pylori*–related gastric cancer. The aim of this study was to evaluate the role of proinflammatory cytokine gene polymorphisms in gastric and esophageal cancers defined by anatomic subsite.** ***Methods:*** **We assessed polymorphisms of the *IL-1* gene cluster and 4 other cytokine genes in a population-based case-control study of upper gastrointestinal cancers, including gastric cardia (n = 126) and noncardia adenocarcinoma (n = 188), esophageal squamous cell carcinoma (n = 53), and adenocarcinoma (n = 108), and frequency-matched controls (n = 212). ORs for the different cancers were computed from logistic regression models adjusted for potential confounding factors.** ***Results:*** **Proinflammatory genotypes of tumor necrosis factor α and *IL-10* were each associated with more than doubling of the risk of noncardia gastric cancer. Carriage of multiple proinflammatory polymorphisms of IL-1B$_o$, IL-1 receptor antagonist, tumor necrosis factor A, and IL-10 conferred greater risk, with ORs (and 95% confidence intervals) of 2.8 (1.6–5.1) for one, 5.4 (2.7–10.6) for 2, and 27.3 (7.4–99.8) for 3 or 4 high-risk genotypes. In contrast, these polymorphisms were not consistently related to the risks of esophageal or gastric cardia cancers. Polymorphisms in *IL-4* and *IL-6* were not associated with any of the cancers studied.** ***Conclusions:*** **A proinflammatory cytokine genetic profile increases the risk of noncardia gastric adenocarcinoma but not other upper gastrointestinal cancers, possibly by inducing a hypochlorhydric and atrophic response to gastric *H. pylori* infection.**

Cancers of the upper gastrointestinal tract are collectively the second most common type of cancer worldwide[1] and comprise 4 distinct entities: squamous cell carcinoma of the esophagus, adenocarcinoma of the esophagus, adenocarcinoma of the gastric cardia, and adenocarcinoma of the distal (noncardia) stomach. The available evidence for these tumors suggests that different pathogenic mechanisms are involved. For example, noncardia gastric adenocarcinoma occurs excessively in patients with gastric atrophy and hypochlorhydria,[2] which seem to protect against the more proximal cardia and esophageal adenocarcinomas.[3] Time trends also differ; the incidence rates for adenocarcinomas of the esophagus and gastric cardia have increased steeply in industrialized countries since the mid-1970s, while rates for esophageal squamous cell carcinoma and noncardia gastric adenocarcinoma have remained stable or decreased.[4,5] The decline in incidence of noncardia gastric adenocarcinoma has paralleled the decreasing prevalence of infection with *Helicobacter pylori*, which plays an essential role in initiating this cancer[6] but could possibly protect against the more proximal cancers.[7,8]

Host genetic factors are emerging as key determinants of disease risk for many cancers.[9,10] We recently reported that functional polymorphisms in the genes for interleukin 1β (*IL-1B*) and its endogenous receptor antagonist (*IL-1RN*) were associated with increased risk of gastric cancer and its precursors.[11,12] These polymorphisms have been shown to significantly affect gastric mucosal IL-1β production in response to *H. pylori* infection.[13] The effect of these polymorphisms is therefore most likely mediated

Abbreviations used in this paper: CI, confidence interval; IL, interleukin; IL-1RN, interleukin-1 receptor antagonist; OR, odds ratio; TNF, tumor necrosis factor.

0016-5085/03/$30.00
doi:10.1016/S0016-5085(03)00157-4

Table 1. Sequences of Primers and Probes Used in the 5′ Nuclease (TaqMan) Assays

Gene	Primers	Probes
IL-1B-511	F: 5′-TCCTCAGAGGCTCCTGCAAT-3′	FAM:TGTTCTCTGCCTCGGGAGCTCTCTG
C/T	R: 5′-TGTGGGTCTCTACCTTGGGTG-3′	VIC:CTGTTCTCTGCCTCAGGAGCTCTCTGTC
IL-4-590	F: 5′-CCTGTCCTTCTCAAAACACTAAACTTG-3′	FAM:AACATTGTCCCCCAGTGCTGGG
C/T	R: 5′-GCAGAATAACAGGCAGACTCTCCTA-3′	VIC:AGAACATTGTTCCCCAGTGCTGGG
IL-6-174	F: 5′-CAATGACGACCTAAGCTGCACT-3′	FAM:AGTTGTGTCTTGCGATGCTAAAGGACG
G/C	R: 5′-GCTGATTGGAAACCTTATTAAGATTGT-3′	VIC:AGTTGTGTCTTGCCATGCTAAAGGACG
IL-10-1082	F: 5′-CACACACACACAAATCCAAGACAA-3′	FAM:AAGGCTTCTTTGGGAGGGGGAAGTAG
G/C	R: 5′-GCTGGATAGGAGGTCCCTTACTTT-3′	VIC:AAGGCTTCTTTGGGAAGGGGAAGTAGG
IL-10-819	F: 5′-GGGTGAGGAAACCAAATTCTCA-3′	FAM:TACAGGTGATGTAACATCTCTGTGCCTCAG
C/T	R: 5′-CATGACCCCTACCGTCTCTATTTTA-3′	VIC:TGTACAGGTGATGTAATATCTCTGTGCCTCAGT
IL-10-592	F: 5′-GGTAAAGGAGCCTGGAACACATC-3′	FAM:ACCCCGCCTGTCCTGTAGGAAGC
C/A	R: 5′-CCAAGCAGCCCTTCCATTT-3′	VIC:ACCCCGCCTGTACTGTAGGAAGC
TNF-A-308	F: 5′-CCCCAAAAGAAATGGAGGC-3′	FAM:AGGGGCATGGGGACGGG
G/A	R: 5′-TCTTCTGGGCCACTGACTGAT-3′	VIC:TGAGGGGCATGAGGACGGG

F, forward primer; R, reverse primer; FAM, wild-type allele; VIC, mutant allele.

through the higher production of IL-1β, which is an important proinflammatory cytokine and a powerful inhibitor of acid secretion.[14] However, our initial study only addressed gastric cancer, which consisted predominately of the noncardia gastric tumors.

The genetic control of inflammation may affect other upper gastrointestinal cancers as well. Both proinflammatory and anti-inflammatory cytokine genes have recognized polymorphisms that alter gene transcription and/or expression.[15] In addition to the *IL-1* gene cluster, candidate genes include those encoding the proinflammatory cytokines tumor necrosis factor α (TNF-α) and IL-6, as well as the anti-inflammatory IL-4 and IL-10. We therefore undertook a study to further investigate the relationship between inflammatory cytokine polymorphisms and the risk of upper gastrointestinal tract cancers.

Materials and Methods

Study Population

The subjects in this study came from a multicenter, case-control study of esophageal and gastric cancer conducted in 3 geographic areas of the United States with population-based tumor registries.[16] Population-based controls were frequency matched by 5-year age group and sex, and were selected by random-digit dialing and Health Care Financing Administration roster sampling as previously described.[16] Genomic DNA was obtained from peripheral blood samples that had been collected at some of the participating centers[17] or from paraffin-embedded tissue sections. DNA samples were available for 161 subjects with esophageal cancer (53 with squamous cell carcinoma and 108 with adenocarcinoma), 314 subjects with gastric adenocarcinoma (126 with cardia and 188 with noncardia adenocarcinomas), and 212 controls. *H. pylori* serologic status was available on 214 subjects (46%) with cancer and all controls as previously reported.[17]

Genotyping

We assessed a total of 7 single nucleotide polymorphisms in *IL-1B* (−511 C/T), *IL-4* (−590 C/T), *IL-6* (−174 G/C), *IL-10* (−1082 G/A, −819 C/T, −592 C/A), and *TNF-A* (−308 G/A), as well as the variable number of tandem repeat polymorphism of *IL-1RN*. Single nucleotide polymorphisms were discriminated by 5′ nuclease polymerase chain reaction assays (TaqMan) using methods as previously described.[11] The sequences of the primers and probes used in the TaqMan assays are provided in Table 1 and were all developed in our laboratory except for *IL-4*-590 (C/T), which was previously reported by Holloway et al.[18]

For the *IL-1RN* variable number of tandem repeat polymorphism, genomic DNA was polymerase chain reaction amplified and resultant products sized by gel electrophoresis as previously described[11]; the rarer alleles 3 and 4 were grouped in the statistical analysis.

Genotype data were complete for the *IL-1B*, *IL1-RN*, *IL-10*, and *TNF-A* markers in all of the cases and in 210 controls; 2 control samples with degraded DNA could not be typed completely and were excluded from analysis. The *IL-4* and *IL-6* polymorphisms were only genotyped for subjects with peripheral blood DNA because tissue DNA samples were limited. For 27 subjects with DNA from both blood and tissue samples, genotype results for the other 6 polymorphisms were more than 99% identical in quality control analyses.

Statistical Analysis

Hardy–Weinberg equilibrium of alleles at individual loci was assessed by χ^2 statistics. Odds ratios (OR) with Cornfield 95% confidence intervals (CIs) were computed by logistic regression using STATA version 7.0 software (STATA Press, College Station, TX). ORs for the different cancers were adjusted for age (categorized as younger than 50, 50–59, 60–69, and 70 years or older), sex, and race (categorized as white and all other); alternative analyses stratified by race provided similar estimates. Additional models were adjusted for the effects of the genetic polymorphisms on one another,

Table 2. Selected Characteristics of Patients With Different Types of Upper Gastrointestinal Cancer and Controls

Characteristic	Esophageal cancer		Gastric cancer		
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)	Controls (n = 210)
Age (*median years*)	66	65	66	70	66
Sex (*% male*)	89	86	87	77	85
Race (*% white*)	75	97	97	85	94
Body mass index (*median kg/m²*)	24.3	26.6	25.8	25.1	25.3
Cigarette Users (%)					
Current	47	32	33	27	24
Former	43	47	47	48	45
Alcohol (*% regular users*)	98	76	80	70	85
History of reflux symptoms (%)	21	61	40	38	30
History of peptic ulcer (%)					
Gastric	13	11	10	19	7
Duodenal	4	10	9	12	6
Family history of cancer (%)					
Esophageal	0	2	2	2	2
Gastric	8	8	8	14	4
Lauren classification (%)					
Diffuse (%)	—	14	24	47	—
Intestinal (%)	—	73	59	34	—
H. pylori immunoglobulin G antibody (*% positive*)	58	32	26	48	40
DNA source (*% peripheral blood*)	45	61	46	35	100

histologic subtype (Lauren classification), cigarette smoking (categorized as current, former, and never), alcohol consumption (categorized as greater or less than one drink per month), history of gastroesophageal reflux disease,[19] body mass index (in quartiles), history of gastric or duodenal ulcer disease, *H. pylori* serologic status,[17] and family history of esophageal or gastric cancer. Population-attributable fractions of the various cancers were estimated from logistic regression models comparing subjects with one or more polymorphisms to subjects with none.

The study was approved by the institutional review boards of the participating centers, and written informed consent was obtained from all subjects.

Results

Demographic and risk-factor characteristics of the subjects with cancer and the controls (Table 2) were similar to those reported in the previous study.[16]

Among controls, the alleles at all of the individual loci studied were in Hardy–Weinberg equilibrium, with nominally nonsignificant χ^2 values (Table 3). Nevertheless, only 9 (4%) had the homozygous variant genotype of *IL-1B*-511, whereas 16 (7%) were expected based on allele frequencies (Hardy–Weinberg $\chi^2 = 5.6$; $P = 0.07$, 2 *df*). Based on genotype frequencies reported from other white populations, the paucity of *IL-1B*-511T/T among the controls was an anomaly that had the effect of exaggerating the case-control comparisons for this genotype. In all subjects, there was 100% linkage disequilibrium between the *IL-10*-819 and -592 alleles as previously reported.[20,21]

Associations With Polymorphisms in the *IL-1* Gene Cluster

Carriers of the proinflammatory *IL-1B*-511T allele had a significantly increased risk of noncardia gastric cancer; the association seemed to be greater in homozygotes than in heterozygotes due to the low frequency of homozygous controls (Table 4). In a logistic regression model that included the other genetic markers, the adjusted OR for *IL-1B*-511T+ carriers (homozygotes and heterozygotes) was 2.3 (95% CI, 1.4–3.8). Although homozygotes also had statistically significant excess risks of the other 3 cancers, these increases may have been artifactual because there were no increases in risk for heterozygotes or for *IL-1B*-511T+ carriers overall (Table 5).

Additionally, *IL-1RN**2 was associated with an increased risk of both noncardia gastric cancer and esophageal squamous cell cancer but only in homozygotes, with adjusted ORs of 3.6 (95% CI, 1.7–7.6) and 4.2 (95% CI, 1.3–13.9), respectively (Table 5). Carriers of the rarer *IL-1RN* alleles 3 or 4 had an increased risk of esophageal adenocarcinoma of marginal statistical significance ($P = 0.03$).

Table 3. Cytokine Genotype Frequencies in Patients With Different Types of Upper Gastrointestinal Cancer and Controls

Genotype	Esophageal cancer (%)		Gastric cancer (%)		Controls (%) (n = 210)
	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)	
IL-1B-511					
C/C	21 (40)	50 (46)	57 (45)	47 (25)	104 (50)
C/T	21 (40)	46 (43)	56 (44)	98 (52)	97 (46)
T/T	11 (20)	12 (11)	13 (11)	43 (23)	9 (4)
IL-1RN					
1/1	30 (57)	49 (46)	69 (55)	81 (43)	114 (55)
1/2	12 (23)	40 (37)	42 (33)	59 (31)	76 (36)
1/3,4	2 (3)	10 (9)	3 (2)	2 (1)	7 (3)
2/2	8 (15)	8 (7)	12 (10)	45 (24)	13 (6)
2/3	1 (2)	1 (1)	0 (0)	1 (1)	0 (0)
IL-4-590					
G/G	11 (46)	44 (67)	41 (71)	37 (58)	153 (73)
G/T	11 (46)	17 (26)	16 (27)	21 (33)	46 (22)
T/T	2 (8)	5 (7)	1 (2)	6 (9)	10 (5)
IL-6-174					
G/G	13 (54)	20 (30)	22 (38)	33 (51)	83 (40)
G/C	6 (25)	33 (50)	31 (53)	21 (32)	98 (47)
C/C	5 (21)	13 (20)	5 (9)	11 (17)	28 (13)
IL-10-1082					
G/G	9 (17)	23 (21)	30 (24)	31 (16)	48 (23)
G/A	28 (53)	59 (55)	62 (49)	71 (38)	103 (49)
A/A	16 (30)	26 (24)	34 (27)	86 (46)	59 (28)
IL-10-592					
C/C	35 (66)	72 (67)	80 (64)	98 (52)	127 (61)
C/A	15 (28)	29 (27)	38 (30)	63 (34)	70 (33)
A/A	3 (6)	7 (6)	8 (6)	27 (14)	13 (6)
IL-10					
GCC/ GCC	8 (15)	23 (21)	30 (24)	31 (16)	48 (23)
Other[a]	43 (81)	79 (73)	88 (70)	131 (70)	150 (71)
ATA/ATA	2 (4)	6 (6)	8 (6)	26 (14)	12 (6)
TNF-A-308					
G/G	41 (77)	81 (75)	91 (72)	110 (59)	152 (72)
G/A	10 (19)	24 (22)	30 (24)	57 (30)	52 (25)
A/A	2 (4)	3 (3)	5 (4)	21 (11)	6 (3)

[a]*IL-10* genotypes other than GCC/GCC or ATA/ATA.

Associations With Polymorphisms in Other Proinflammatory Cytokine Genes

Carriers of the proinflammatory *TNF-A*-308A allele had a significantly increased risk of noncardia gastric cancer that was more pronounced for homozygotes than heterozygotes. Adjusted for the effects of the other genetic markers, the OR for *TNF-A*-308A+ was 2.2 (95% CI, 1.4–3.7). *TNF-A*-308A+ carriers were not at increased risk for the other gastrointestinal cancers (Table 5). No statistically significant associations of *IL-6*-174C were seen for any of the 4 cancers.

Associations With Polymorphisms in Anti-inflammatory Cytokine Genes

Subjects homozygous for the hypoactive IL-10 alleles *IL-10*-1082A and *IL-10*-592A, as well as for the "low IL-10" haplotype ATA (*IL-10*-1082A, *IL-10*-819T, and *IL-10*-592A)[21] had significantly increased risks of noncardia gastric cancer. Compared with homozygotes for the "high IL-10" haplotype GCC, the adjusted OR for low IL-10 homozygotes was 2.5 (95% CI, 1.1–5.7). The low IL-10 haplotype was not associated with risk for the other cancers (Table 5). Homozygotes for the *IL-4*-590T allele were not at altered risk for any of the cancers, although heterozygotes had an excess of esophageal squamous cell cancer of marginal statistical significance (P = 0.02; Table 4).

Association With Composite of Cytokine Polymorphisms

To analyze the combined association with increases in proinflammatory cytokines and decreases in

Table 4. Age-, Sex-, and Race-Adjusted ORs (and Cornfield 95% CIs) for the Association of Cytokine Gene Polymorphisms With Different Types of Upper Gastrointestinal Cancer

	Esophageal cancer		Gastric cancer	
Genotype	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)
IL-1B-511				
C/C	1.0	1.0	1.0	1.0
C/T	0.9 (0.5–1.9)	1.0 (0.6–1.6)	1.1 (0.7–1.8)	2.4 (1.5–3.8)
T/T	6.6 (2.2–19.8)	2.93 (1.1–7.7)	3.1 (1.2–8.0)	9.5 (4.0–22.7)
IL-1RN				
1/1	1.0	1.0	1.0	1.0
1/2	0.7 (0.3–1.6)	1.2 (0.7–2.0)	0.9 (0.5–1.4)	1.2 (0.7–1.9)
1/3,4	2.1 (0.4–12.0)	3.3 (1.1–9.5)	0.7 (0.2–2.9)	0.8 (0.2–4.0)
2/2	3.3 (1.2–9.2)	1.3 (0.5–3.5)	1.6 (0.7–3.7)	5.4 (2.6–11.1)
IL-4-590				
G/G	1.0	1.0	1.0	1.0
G/T	3.2 (1.2–8.3)	1.4 (0.7–2.7)	1.4 (0.7–2.9)	1.8 (0.9–3.5)
T/T	1.7 (0.2–14.2)	2.0 (0.5–7.6)	0.6 (0.1–4.9)	1.4 (0.3–5.9)
IL-6-174				
G/G	1.0	1.0	1.0	1.0
G/C	0.5 (0.2–1.5)	1.4 (0.8–2.8)	1.1 (0.6–2.1)	0.7 (0.3–1.3)
C/C	1.2 (0.4–4.1)	1.9 (0.8–4.4)	0.6 (0.2–1.8)	1.3 (0.5–3.1)
IL-10-1082				
G/G	1.0	1.0	1.0	1.0
G/A	1.3 (0.5–3.0)	1.2 (0.7–2.2)	1.0 (0.6–1.8)	1.0 (0.6–1.8)
A/A	1.1 (0.4–2.9)	0.8 (0.4–1.7)	1.1 (0.6–2.1)	1.9 (1.1–3.5)
IL-10-592				
C/C	1.0	1.0	1.0	1.0
C/A	0.6 (0.3–1.2)	0.8 (0.5–1.3)	0.9 (0.6–1.5)	1.2 (0.8–1.9)
A/A	0.3 (0.1–1.4)	1.3 (0.5–3.8)	1.4 (0.5–3.9)	2.4 (1.1–5.1)
IL-10				
GCC/GCC	1.0	1.0	1.0	1.0
Other[a]	1.5 (0.7–3.6)	1.1 (0.6–1.9)	1.0 (0.6–1.7)	1.2 (0.7–2.1)
ATA/ATA	1.0 (0.1–6.0)	1.2 (0.3–4.3)	1.7 (0.5–6.1)	3.4 (1.3–8.8)
TNF-A-308				
G/G	1.0	1.0	1.0	1.0
G/A	0.8 (0.4–1.7)	0.9 (0.5–1.6)	1.0 (0.6–1.6)	1.5 (0.9–2.4)
A/A	1.3 (0.2–6.9)	1.2 (0.3–5.3)	1.5 (0.4–5.0)	4.8 (1.8–12.8)

[a]*IL-10* genotypes other than GCC/GCC or ATA/ATA.

anti-inflammatory cytokines, subjects were classified according to their number of inflammation-promoting cytokine polymorphisms, defined as follows: (1) carriage of *IL-1B*-511T, (2) homozygosity for *IL-1RN**2, (3) carriage of *TNF-A*-308A, and (4) homozygosity for the low IL-10 haplotype ATA. The OR for noncardia gastric cancer increased progressively with increasing number of proinflammatory genotypes to 27.3 (95% CI, 7.4–99.8; Table 6) for 3 or 4 polymorphisms. In contrast, the number of proinflammatory cytokine polymorphisms did not significantly affect the risk of esophageal squamous cell carcinoma, adenocarcinoma, or gastric cardia cancer,

Table 5. Multivariate Model–Estimated ORs (and Cornfield 95% CIs) for the Association of Proinflammatory Genotypes With Different Types of Upper Gastrointestinal Cancer

	Esophageal cancer		Gastric cancer	
Genotype	Squamous cell carcinoma (n = 53)	Adenocarcinoma (n = 108)	Cardia (n = 126)	Noncardia (n = 188)
IL-1B-511T+	0.9 (0.4—2.0)	1.0 (0.6–1.7)	1.2 (0.7–1.9)	2.3 (1.4–3.8)
*IL-1RN**2/*2	4.2 (1.3–13.9)	1.0 (0.4–2.8)	1.4 (0.6–3.3)	3.6 (1.7–7.6)
IL-10 ATA/ATA	0.3 (0.1–2.1)	1.5 (0.5–4.4)	1.3 (0.5–3.6)	2.5 (1.1–5.7)
TNF-A-308A+	0.8 (0.4–1.7)	1.0 (0.6–1.8)	1.1 (0.6–1.8)	2.2 (1.4–3.7)

Table 6. Frequencies and Age-, Sex-, and Race-Adjusted ORs (and Cornfield 95% CIs) for the Association of 1–4 Proinflammatory Polymorphisms in *IL-1B*, *IL-1RN*, *IL-10*, and *TNF-A* With Noncardia Gastric Cancer

Polymorphisms	Cases (n = 188)	Controls (n = 210)	OR (95% CI)
0	22	75	(Referent)
1	74	85	2.8 (1.6–5.1)
2	62	46	5.4 (2.7–10.6)
3	28	4	26.3 (7.1–97.1)
4	2	0	∞ (Undefined)

with ORs for carriage of 3 or 4 proinflammatory genotypes of 0 (95% CI, 0–10.5), 1.3 (95% CI, 0.2–8.0), and 3.6 (95% CI, 0.8–16.7), respectively.

Sixteen percent of the subjects with noncardia gastric cancer but only 2% of the controls had 3 or 4 high-risk genotypes, whereas 37% of the controls compared with 12% of the subjects with noncardia gastric cancer had no high-risk genotypes. Assuming these associations are causal, the combined population-attributable fraction of noncardia gastric cancer due to the effect of these alleles is estimated to be 67%.

Similar results were obtained after exclusion of subjects with DNA from tumor specimens and in separate analyses for each histologic subtype defined by Lauren classification. The estimated effects of the proinflammatory genotypes were not altered substantially after adjustment for the effects of possible confounders of the risk of noncardia gastric cancer (data not shown).

Effects of Cytokine Polymorphisms in *H. pylori*–Seropositive Subjects

Less than half of the subjects with cancer had blood available for *H. pylori* serologic analysis. Among those with *H. pylori* seropositivity, the OR estimates for noncardia gastric cancer were 4.2 for *IL-1B*-511*T, 1.6 for *IL-1RN**2*2, 2.9 for *IL-10* ATA/ATA, and 2.9 for *TNF-A*-308*2. For those with CagA-positive serology, the OR estimates were higher (9.9, 2.9, 5.2, and 6.2 for the above markers, respectively). For the composite estimates, the *H. pylori*–seropositive and CagA-seropositive subjects had OR estimates of 5.4 and 6.6 for one marker, 8.3 and 19.5 for 2 markers, and 42.0 and undefined for 3 markers, respectively. *H. pylori* serologic markers did not apparently affect the OR estimates for the other upper gastrointestinal cancers.

Discussion

We have identified a proinflammatory profile of genetic polymorphisms in *IL-1B*, *IL-1RN*, *IL-10*, and *TNF-A* associated with an increased risk of gastric cancer that was limited to noncardia tumors. No associations were seen with cancers of the gastric cardia or esophagus. The *IL-1* gene cluster polymorphisms were associated with gastric cancer in 2 previous reports.[11,22] The ORs are similar in all 3 studies (all in predominantly white populations), underscoring the central role of IL-1β in the pathogenesis of *H. pylori*–associated gastric cancer. A novel finding in our study is the identification of the proinflammatory genotypes of *TNF-A* and *IL-10* as additional risk factors.

TNF-α is up-regulated early in *H. pylori* colonization and induces the transcription of a wide range of other proinflammatory cytokines and chemokines, amplifying the inflammatory cascade against the infection.[23] Although not as potent as IL-1β, TNF-α also inhibits gastric acid secretion,[24] which paradoxically promotes spread of the organism. The association with the low IL-10 haplotype may be related to the role of IL-10 as an anti-inflammatory cytokine that down-regulates IL-1β, TNF-α, interferon gamma, and other proinflammatory cytokines. Relative deficiency of IL-10 may result in a Th-1–driven hyperinflammatory response to *H. pylori* with greater damage to the gastric mucosa.[25–28] Imbalances among proinflammatory and anti-inflammatory mediators and acid production may favor the development of gastric atrophy and its progression to cancer.

The association of these polymorphisms with gastric cancer requires the presence of *H. pylori* and may be most important early in the disease process. When *H. pylori* infects gastric mucosa, it induces a vigorous inflammatory response with high levels of IL-1β and TNF-α. The direct effects of these cytokines are advantageous to eradication of *H. pylori* organisms, but the concomitant inhibition of acid secretion may extend the area of colonization affecting the corpus mucosa, which is otherwise protected by an acidic environment. The critical role of acid in determining the pattern of gastritis is well shown by the changes resulting from its pharmacologic inhibition. *H. pylori*–infected patients on long-term proton pump inhibitor therapy may experience an exacerbation of gastritis, with a shift to corpus predominance and an increased risk of gastric atrophy.[29]

In addition, a decreased flow of gastric secretions may promote accumulation of the genotoxic by-products of inflammation, causing more mucosal damage and an increased rate of mutation. As the inflammatory process extends across the corpus, the acid secretion is further inhibited in a continuing cycle that accelerates glandular loss and onset of gastric atrophy. The hypochlorhydric

milieu also promotes growth of non–*H. pylori* bacteria, which may contribute to mucosal damage and/or production of carcinogenic *N*-nitrosocompounds.[30] With progression from mild gastritis through severe gastritis, atrophy, and intestinal metaplasia, it becomes more difficult for *H. pylori* to colonize and be detected.[31]

While *H. pylori* infection and host genetics interact to initiate a hypochlorhydric and atrophic phenotype, other factors may contribute to subsequent neoplastic transformation, which does not depend on continued presence of the infection. Diet may be particularly relevant, because greater consumption of fresh fruits and vegetables has been shown to protect against the risk of gastric cancer. In particular, dietary vitamin C reduces the formation of *N*-nitrosocompounds and scavenges mutagenic reactive oxygen metabolites generated by gastric inflammation,[32] and supplemental vitamin C was associated with a significantly lower risk of noncardia gastric cancer in this study population[33]; furthermore, vitamin C concentrations and bioavailability are reduced in the presence of *H. pylori* infection.[34,35] Another factor for transformation is cigarette smoking, which was found to nearly double the risk of transition from atrophic gastritis to dysplasia in a high-risk population.[36] Thus, cytokine gene polymorphisms represent one component of a complex interplay among the host, pathogen, and environmental factors involved in gastric carcinogenesis.

In the present study, we did not have an opportunity to fully assess the interaction between host genetic factors and *H. pylori* virulence factors, because gastric biopsy material for isolation of *H. pylori* strains was not available. Our limited analysis using the subgroup of subjects with serologic data showed a strong effect of the proinflammatory cytokine gene polymorphisms in the presence of CagA seropositivity. Our data thus concur with the recent report by Figueiredo et al.[37] that the risk of gastric cancer is greatest in subjects with both bacterial and host high-risk genotypes.

Notably, the proinflammatory cytokine profile did not affect the risk of the other upper gastrointestinal tract cancers. Esophageal adenocarcinoma is believed to arise from Barrett's esophagus, a precancerous metaplasia induced by chronic gastroesophageal reflux of acidic gastric contents.[38–40] Theoretically, *H. pylori*–induced gastritis and secondary hypochlorhydria reduce the amount of acid production and refluxate. Accordingly, it has been argued that *H. pylori* colonization,[41] particularly with the CagA-positive strains, may be protective against gastroesophageal reflux disease and its complications, such as Barrett's esophagus, dysplasia, and adenocarcinoma.[7,17,42,43] It might be expected that proinflammatory genotypes enhancing gastric acid inhibition would be protective against esophageal and possibly gastric cardia adenocarcinoma, but our data do not provide support for this hypothesis.

Our study also showed that homozygous carriage of the proinflammatory allele of *IL-1RN* was associated with risk of esophageal squamous cell carcinoma. However, risk was not related to the other proinflammatory markers associated with response to *H. pylori*, suggesting differences in the cytokine repertoire predisposing to the distinctive etiologic factors for esophageal squamous cell cancer, including alcohol, smoking, and nutritional deficiency. The associations we observed with *IL-1RN* and *IL-4* polymorphisms warrant further investigation in larger studies of esophageal cancer.

Although required for inclusion in this study, DNA collection was unlikely to have been influenced by cytokine genotype and therefore should not have biased our results. The absence of selection or survival bias was further supported by the similarity in genotyping findings based on subjects with DNA from blood or from tumor tissue. In addition, there was no evidence that DNA availability for the present study was associated with demographic or risk-factor characteristics previously reported for these populations.[16]

In conclusion, our findings indicate that a proinflammatory host genotype favors the development of a hypochlorhydric, atrophic response to gastric infection with *H. pylori*, which in turn predisposes to noncardia gastric adenocarcinoma but not to cardia or esophageal cancers. Pathophysiologic considerations indicate that cytokine dysregulation is important in initiating this disease process, but the multistep progression from atrophy to neoplastic transformation depends on other factors, including diet and tobacco. Better understanding of the risk factors involved in the early and late stages of gastric carcinogenesis should enhance the prospects for preventive and therapeutic interventions.

References

1. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80:827–841.
2. McColl KE, El-Omar E, Gillen D. *Helicobacter pylori* gastritis and gastric physiology. Gastroenterol Clin North Am 2000;29:687–703.
3. Blaser MJ. Hypothesis: the changing relationships of *Helicobacter pylori* and humans: implications for health and disease. J Infect Dis 1999;179:1523–1530.
4. Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 1991;265:1287–1289.
5. Devesa SS, Blot WJ, Fraumeni JF Jr. Changing patterns in the

incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83:2049–2053.
6. Webb PM, Forman D. *Helicobacter pylori* as a risk factor for cancer. Baillieres Clin Gastroenterol 1995;9:563–582.
7. Peek RM Jr, Vaezi MF, Falk GW, Goldblum JR, Perez-Perez GI, Richter JE, Blaser MJ. Role of *Helicobacter pylori* cagA(+) strains and specific host immune responses on the development of premalignant and malignant lesions in the gastric cardia. Int J Cancer 1999;82:520–524.
8. Peek RM Jr, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002;2:28–37.
9. Ponder BA. Cancer genetics. Nature 2001;411:336–341.
10. Peto J, Houlston RS. Genetics and the common cancers. Eur J Cancer 2001;37(Suppl 8):S88–S96.
11. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398–402.
12. Furuta T, El-Omar EM, Xiao F, Shirai N, Takashima M, Sugimurra H. Interleukin 1β polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. Gastroenterology 2002;123:92–105.
13. Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1β production in *Helicobacter pylori* infection. Gastroenterology 2002;123:1793–1803.
14. El-Omar EM. The importance of interleukin 1beta in *Helicobacter pylori* associated disease. Gut 2001;48:743–747.
15. Bidwell JL, Keen LJ, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C, D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. Available at: http://bris.ac.uk/pathandmicro/services/GAI/cytokine4.htm. Accessed June 3, 2002.
16. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH, Rotterdam H, West AB, Dubrow R, Stanford JL, Mayne ST, Farrow DC, Niwa S, Blot WJ, Fraumeni JF Jr. Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1997;89:1277–1284.
17. Chow WH, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB, Fraumeni JF Jr. An inverse relation between cagA+ strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res 1998;58:588–590.
18. Holloway JW, Beghe B, Turner S, Hinks LJ, Day IN, Howell WM. Comparison of three methods for single nucleotide polymorphism typing for DNA bank studies: sequence-specific oligonucleotide probe hybridisation, TaqMan liquid phase hybridisation, and microplate array diagonal gel electrophoresis (MADGE). Hum Mutat 1999;14:340–347.
19. Farrow DC, Vaughan TL, Sweeney C, Gammon MD, Chow WH, Risch HA, Stanford JL, Hansten PD, Mayne ST, Schoenberg JB, Rotterdam H, Ahsan H, West AB, Dubrow R, Fraumeni JF Jr, Blot WJ. Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. Cancer Causes Control 2000;11:231–238.
20. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1–8.
21. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999;42:1101–1108.
22. Machado JC, Pharoah P, Sousa S, Carvalho R, Oliveira C, Figueiredo C, Amorim A, Seruca R, Caldas C, Carneiro F, Sobrinho-Simoes M. Interleukin 1β and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology 2001;121:823–829.
23. Genta RM. The immunobiology of *Helicobacter pylori* gastritis. Semin Gastrointest Dis 1997;8:2–11.
24. Beales IL, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. Gut 1998;42:227–234.
25. Eaton KA, Mefford M, Thevenot T. The role of T cell subsets and cytokines in the pathogenesis of *Helicobacter pylori* gastritis in mice. J Immunol 2001;166:7456–7461.
26. Chen W, Shu D, Chadwick VS. Reduced colonization of gastric mucosa by *Helicobacter pylori* in mice deficient in interleukin-10. J Gastroenterol Hepatol 2001;16:377–383.
27. Bodger K, Crabtree JE. *Helicobacter pylori* and gastric inflammation. Br Med Bull 1998;54:139–150.
28. Berg DJ, Lynch NA, Lynch RG, Lauricella DM. Rapid development of severe hyperplastic gastritis with gastric epithelial dedifferentiation in *Helicobacter* felis-infected IL-10(−/ −) mice. Am J Pathol 1998;152:1377–1386.
29. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, Havu N, Festen HP, Liedman B, Lamers CB, Jansen JB, Dalenback J, Snel P, Nelis GF, Meuwissen SG. Atrophic gastritis and *Helicobacter pylori* infection in patients with reflux esophagitis treated with omeprazole or fundoplication. N Engl J Med 1996;334:1018–1022.
30. Sanduleanu S, Jonkers D, De Bruine A, Hameeteman W, Stockbrugger RW. Double gastric infection with *Helicobacter pylori* and non-*Helicobacter pylori* bacteria during acid-suppressive therapy: increase of pro-inflammatory cytokines and development of atrophic gastritis. Aliment Pharmacol Ther 2001;15:1163–1175.
31. Kuipers EJ. Review article: exploring the link between *Helicobacter pylori* and gastric cancer. Aliment Pharmacol Ther 1999;13(Suppl 1):3–11.
32. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52:6735–6740.
33. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, West AB, Rotterdam H, Blot WJ, Fraumeni JF Jr. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 2001;10:1055–1062.
34. Banerjee S, Hawksby C, Miller S, Dahill S, Beattie AD, McColl KE. Effect of *Helicobacter pylori* and its eradication on gastric juice ascorbic acid. Gut 1994;35:317–322.
35. Woodward M, Tunstall-Pedoe H, McColl K. *Helicobacter pylori* infection reduces systemic availability of dietary vitamin C. Eur J Gastroenterol Hepatol 2001;13:233–237.
36. Kneller RW, You WC, Chang YS, Liu WD, Zhang L, Zhao L, Xu GW, Fraumeni JF Jr, Blot WJ. Cigarette smoking and other risk factors for progression of precancerous stomach lesions. J Natl Cancer Inst 1992;84:1261–1266.
37. Figueiredo C, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, Van Doorn LJ, Carneiro F, Sobrinho-Simoes M. *Helicobacter pylori* and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. J Natl Cancer Inst 2002;94:1680–1687.
38. Fitzgerald RC, Omary MB, Triadafilopoulos G. Dynamic effects of acid on Barrett's esophagus. An ex vivo proliferation and differentiation model. J Clin Invest 1996;98:2120–2128.
39. Fitzgerald RC, Omary MB, Triadafilopoulos G. Altered sodium-hydrogen exchange activity is a mechanism for acid-induced hy-

perproliferation in Barrett's esophagus. Am J Physiol 1998;275: G47–G55.
40. Niemantsverdriet EC, Timmer R, Breumelhof R, Smout AJ. The roles of excessive gastro-esophageal reflux, disordered esophageal motility and decreased mucosal sensitivity in the pathogenesis of Barrett's oesophagus. Eur J Gastroenterol Hepatol 1997; 9:515–519.
41. Blaser MJ. In a world of black and white, *Helicobacter pylori* is gray. Ann Intern Med 1999;130:695–697.
42. Vaezi MF, Falk GW, Peek RM, Vicari JJ, Goldblum JR, Perez-Perez GI, Rice TW, Blaser MJ, Richter JE. CagA-positive strains of *Helicobacter pylori* may protect against Barrett's esophagus. Am J Gastroenterol 2000;95:2206–2211.
43. Vicari JJ, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, Perez-Perez GI, Halter SA, Rice TW, Blaser MJ, Richter JE. The seroprevalence of cagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology 1998;115:50–57.

Received December 2, 2002; Accepted January 16, 2003.

Address requests for reprints to: Emad M. El-Omar, M.D., Department of Medicine and Therapeutics, Aberdeen University, Institute of Medical Sciences, Foresterhill, Aberdeen AB25 2ZD, Scotland. e-mail: e.el-omar@abdn.ac.uk; fax: (44) 0-1224-554761.

Supported in part by the National Cancer Institute. E.M.E. received a European *H. pylori* Study Group Research Fellowship from the Digestive Disorders Foundation (United Kingdom).

The authors thank Drs. Heidi Rotterdam and Brian West for reviewing medical records and pathology slides for case eligibility, Dr. Denise Whitby for laboratory support, and Shelley Niwa for computing support.